

Quick Tests for the Determination of Ammonia in Poultry Litter

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ABSTRACT The objective of this work was to test the ability of various available quick tests to determine ammonia concentration of poultry litters. A total of 136 samples was collected from brood chambers of poultry houses. Samples were equally divided between surface samples (top 25 mm) and core samples. Samples were frozen until analysis but received no further processing. Samples were analyzed for ammonia by autoanalyzer (standard) and several quick tests (conductivity, Quantofix N-Volumeter, and Reflectoquant). In addition, samples were analyzed by near-infrared spectroscopy by scanning samples using a large-sample transport device on a FOSS-NIRSystems model 6500 (64 co-added scans from 400 to

2,498 nm). Results showed that, although ammonia could be determined with reasonable accuracy by near-infrared spectroscopy using data in the 1,100 to 2,498 nm spectral range (final calibration R^2 of ~0.90), none of the quick tests, including near-infrared, worked as well as previously found with dairy manures. The best results were found using the Quantofix or Reflectoquant (R^2 of ~0.75), and conductivity worked only with the core samples (R^2 of ~0.75). It is believed that interferences due to the presence of uric acid (spectroscopy, Quantofix, and Reflectoquant) and sodium bisulfate used to treat the litter (conductivity) were the cause of the decreased accuracies as compared to results achieved previously with dairy manures.

(Key words: ammonia, near-infrared, nitrogen, poultry litter, quick test)

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INTRODUCTION

Due to increasing concerns with the environmental effects of animal wastes, there is a need for rapid, inexpensive, and accurate means to analyze these materials. One of the primary compounds of interest is ammonia due to its potential as a source of N fertilizer, environmental pollutant, or odorant. In addition, in poultry houses there is a need to control ammonia volatilization because the litter is allowed to accumulate for several flocks. This control is often achieved by adding compounds such as sodium bisulfate to absorb or react with the ammonia to form nonvolatile forms of N.

At the present time, several so-called quick tests (QTS) are commercially available for ammonia determination. Detailed descriptions of the available QTS have been given previously (Van Kessel et al., 1999). Briefly, the Quantofix-N-Volumeter determines ammonia by measuring the volume of N_2 gas produced through the reaction of ammonia with liquid hypochlorite bleach (ClO^-). The Agros meter is dependent on the same reaction but

uses powdered $CaClO$ and pressure measurements. Ammonia can also be determined by relating the conductivity of the manure to the ammonia concentration with a conductivity pen or meter. Two spectroscopic techniques have also been used. The first, the RQFlex Reflectoquant is a small, portable colorimeter (8.5 by 18 by 2.5 cm) that determines ammonia in solution by reading small test strips (based on a reaction between ammonia and Nessler's reagent) dipped into the test solution. The second spectroscopic method uses near-infrared reflectance spectroscopy (NIRS) to find relationships between spectral absorbances between 400 and 2,498 nm and ammonia in the sample (Marten et al., 1985).

Previous work has demonstrated that NIRS can be used to determine the ammonium-N content of dairy manures (Reeves and Van Kessel, 2000a,b) and poultry manures obtained from deep pits and floor scrapings (Reeves, 2001). Finally, hydrometers have also been used to determine ammonia concentration in manure slurries.

As with any analysis method, the sample in question can greatly affect the accuracy or even the applicability of a testing method. The most obvious example being that a sample needs to be sufficiently liquid for hydrometer

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Abbreviation Key: NIR = near-infrared; NIRS = near-infrared spectroscopy; QTS = quick tests.

readings to be practical. Due to the specific samples and test needs, there are other less obvious problems that can also exist.

The objective of this research was to determine the ability of various available QTS to determine ammonia in poultry litters.

METHODS AND MATERIALS

Samples

Poultry litter samples ($n = 136$) were collected from brood chambers of poultry houses. All litters were obtained from broiler houses of a single company and represent litter from two to 12 flocks on the original bedding base. Samples were equally divided between surface samples (top 25 mm) and core samples (10-cm polyvinyl chloride coring tool). Samples were collected 24 h after application of sodium bisulfate (two levels used based on litter age) at the day of chick placement. Samples were frozen (-20°C) until analysis but were otherwise not processed.

Conventional Assays and QTS

Ammonia concentrations for all samples were determined with a Technicon Autoanalyzer using the hypochlorite-indophenol method (AOAC, 1990). For this analysis, duplicate 2.5-g samples of each litter were shaken with 250 mL of 2 M KCl and 0.1 M HCl for 1 h on a Burrell wrist-action shaker.² Aliquots were then filtered using a serum separator and analyzed within 24 h. DM was determined by drying duplicate subsamples at 100°C to constant weight.

For the conductivity and Reflectoquant measurements, duplicate 2-g samples in 300 mL deionized H_2O were shaken for 30 min on the wrist action shaker. Conductivity measurements were made with a conductivity meter.³ The Reflectoquant system,⁴ consisting of the RQflex Reflectoquant and Reflectoquant test strips, was used for ammonium ion measurements.

NIRS

Samples were scanned in the near-infrared (NIR) by using a large-sample transport on a FOSS NIRSystems model 6500 scanning monochromator.⁵ Samples were thawed and then scanned in polyethylene bags. Samples were scanned from 400 to 2,498 nm at a nominal resolution of 10 nm with data collected every 2 nm for a total of 1,050 spectral data points. Each spectrum consisted of 64 co-added scans with data collected as log (1/reflectance) using a ceramic standard reference spectrum.

All samples were scanned within 24 h of ammonia and DM determinations.

Calibrations and Statistics

Means, other summary statistics, and correlations were computed using SAS software.⁶ Calibrations for all analytes were performed using PLS under GRAMS/32.⁷ A one-out cross validation was performed, and a final calibration was developed and applied to the samples. The number of factors used for any calibration was determined using the F-statistic from the predicted residual sums of squares error. Spectra were pretreated by applying mean and variance scaling or multiplicative scatter correction in addition to a first or second derivative. Calibrations were tested using data from the entire spectral range (400 to 2,498) or data from 1,100 to 2,498 nm only. In addition to calibrations for ammonia, calibrations were also developed for DM and were explored to a limited extent using data from the various QTS.

RESULTS AND DISCUSSION

Analyses

The mean value and range of values for each analyte on an as-is basis are shown in Table 1. The ammonia concentration varied about sixfold, and on average the samples were fairly dry (58 to 87% DM). As a result of the limited range in DM, the composition of the samples was not dramatically different when computed as is, as opposed to on a DM basis. Examination of the QTS data shows that the Quantofix ammonia determinations were on average higher than the autoanalyzer reference method indicated to be present, whereas the Reflectoquant indicated less ammonia than the reference method. Finally, although direct comparison of the conductivity measurements to ammonia are not possible, the range of conductivity (14-fold) when compared to the ammonia range (sixfold) would indicate possible problems with using this QTS.

Compositional data for the core and surface samples are also presented in Table 1. As shown, the core samples had dramatically lower conductivity readings when compared to the surface samples. Previous work with dairy manures (Van Kessel and Reeves, 2000) showed conductivity to be a promising method for estimating manure ammonia concentrations. However, unlike the dairy manures previously studied (Van Kessel and Reeves, 2000), sodium bisulfate had been added to these poultry litters to control ammonia volatilization. Conductivity depends on the number and types of ions present, with H^+ having the highest equivalent ion conductance (Kittsley, 1965). Thus, addition of an acid material such as bisulfate could result in a substantial increase in the overall conductivity. As the H^+ binds with ammonia to form NaNH_4SO_4 or a mixture of Na_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$, the conductivity would decrease. This result could explain why, in this study, the core sample conductivity was, on average, only

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TABLE 1. Composition and quick test results for samples on an as-is basis (mg NH₄-N/g of sample unless otherwise noted)

Assay	N	Mean	SD	Minimum	Maximum
All 136 samples					
% DM (60 C)	136	79.0	5.2	58.2	87.4
Autoanalyzer-NH ₃	136	5.71	1.81	1.94	11.97
Conductivity	136	208.5	82.3	38.0	538.1
Quantofix-N-Meter	136	8.63	2.51	2.02	14.59
Reflectoquant	136	5.17	1.56	2.31	8.95
68 core samples					
% DM (60 C)	68	75.2	4.6	58.2	87.4
Autoanalyzer-NH ₃	68	5.53	1.57	1.94	8.50
Conductivity	68	147.1	39.0	38.0	243.3
Quantofix-N-Meter	68	8.07	2.48	2.02	11.41
Reflectoquant	68	4.89	1.40	2.31	7.80
68 surface samples					
% DM (60 C)	68	82.7	2.3	73.5	87.0
Autoanalyzer-NH ₃	68	5.88	2.02	1.94	11.97
Conductivity	68	269.9	66.9	115.1	538.1
Quantofix-N-Meter	68	9.19	2.43	2.84	14.59
Reflectoquant	68	5.46	1.68	2.44	8.95

one-half that of the surface samples. It should be noted, however, that even for the core samples, the addition of the sodium bisulfate might affect the ability to use conductivity for estimating ammonia concentrations. The amount of added sodium bisulfate would need to be consistent for all samples in order to not introduce variable amounts of conductance to the samples. Overall, however, except for the conductivity measurements, the surface and core samples were not dramatically different in composition, at least by the tests shown here.

QTS Results

Correlations between the reference method and the QTS for all 136 samples are shown in Table 2. Results using conductivity were very poor, unlike previous results using dairy manures ($R^2 = 0.94$; Van Kessel and Reeves, 2000), and were assumed to be due to the presence of the sodium bisulfate, as previously discussed. With a correlation between 0.86 and 0.87, ammonia estimations using the Quantofix (Figure 1) or Reflectoquant (Figure 2) could be useful for rapid analysis in which some error would be acceptable; however, again they were not as

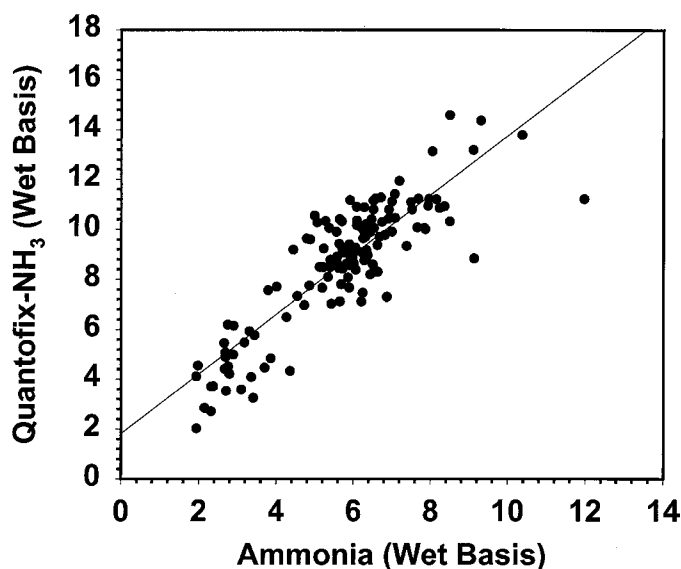
good as previously achieved using dairy manures ($R^2 = 0.97$ and 0.95 for Quantofix and Reflectoquant, respectively). Also, results based on DM (not shown) and as-is computations were essentially the same.

Results using only the core samples were overall improved compared with those using all 136 samples (Table 2). This finding was especially true for conductivity (see also Figure 3). The Quantofix results using only the core samples were about the same as using all 136 samples (Figure 1) or only the surface samples (Table 2). The results using the Reflectoquant, like those for the Quantofix, were very similar for the three sample sets (Table 2).

Overall, from the results presented, it is apparent that the surface samples at times were different from samples obtained deeper in the litter (core samples). Differences in conductivity due to sodium bisulfate addition to trap

TABLE 2. Correlations (r) between composition on an as is basis and quick test results ($P < 0.05$)

Assay	Conductivity	Quantofix	Reflectoquant
All 136 samples			
Autoanalyzer-NH ₃	0.26	0.86	0.87
Conductivity		0.34	0.36
Quantofix			0.83
68 core samples			
Autoanalyzer-NH ₃	0.86	0.90	0.84
Conductivity		0.84	0.79
Quantofix			0.83
68 surface samples			
Autoanalyzer-NH ₃	NS	0.86	0.89
Conductivity		NS	NS
Quantofix			0.83

**FIGURE 1.** Plot (mg NH₄-N/g of sample) of Quantofix results with all 136 samples versus ammonia concentration ($r = 0.86$).

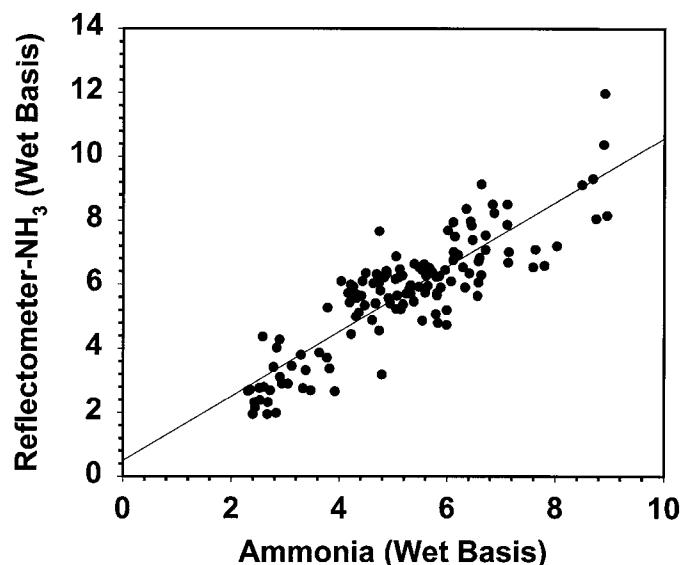


FIGURE 2. Plot (mg $\text{NH}_4\text{-N/g}$ of sample) of Reflectoquant results with all 136 samples versus ammonia concentration ($r = 0.87$).

ammonia may explain the conductivity results, but it appears that other differences may exist between the surface and core samples, which resulted in slightly different correlations for surface versus core samples. Speculation would be that changes in organic fractions, including possibly uric acid, occur as the litter ages and that these changes produce the results observed. From a practical standpoint, the issues are not important in that a test is useful or not for its designed purpose. However, further research may be useful in understanding the basis for the results observed, for modifying the assays used, or for developing new methods.

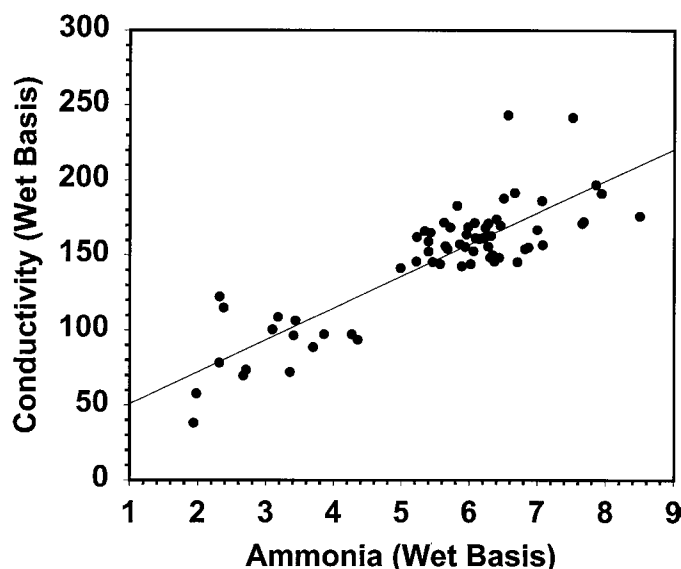


FIGURE 3. Plot of conductivity ($\mu\text{S/cm}$) results with 68 core samples versus ammonia (mg $\text{NH}_4\text{-N/g}$ of sample) concentration ($r = 0.86$).

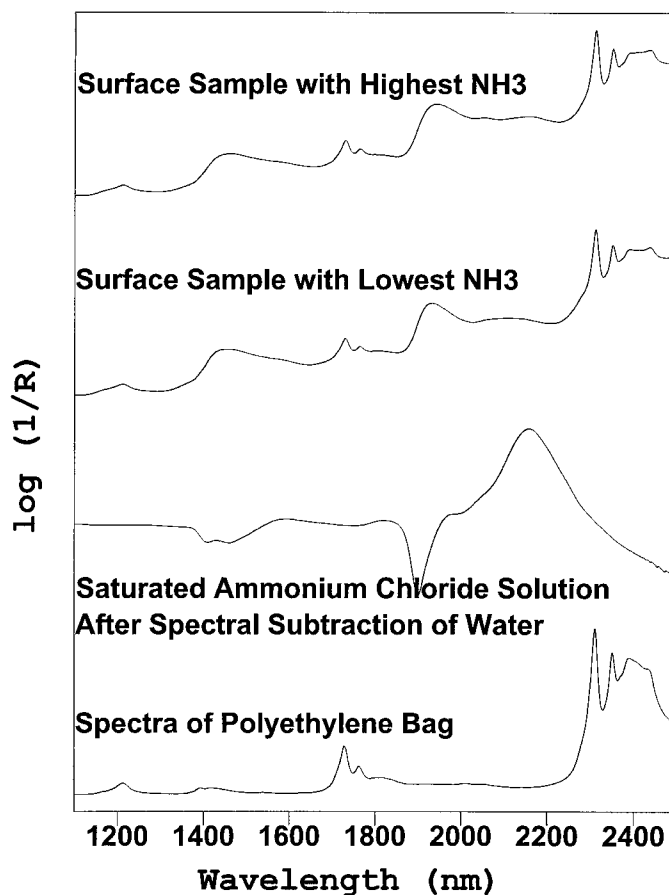


FIGURE 4. Near-infrared spectra of poultry litter with high and low ammonia contents, of saturated ammonium chloride solution (water spectrum subtracted), and of polyethylene sample bag.

Spectra

Figure 4 shows the spectra of some representative samples, a solution of ammonium chloride, and of a polyethylene bag used to hold samples during NIR scanning. The presence of ammonia in solution causes a spectral absorption around 2,150 nm, which could also be observed in the sample spectra. Although crystalline ammonium compounds have absorption bands elsewhere in the NIR spectral range, in solution the band at 2,150 nm has been required for determination of ammonia in manures (Reeves and Van Kessel, 2000b). The sharp bands in the spectra are due to the polyethylene bags that were used to hold the samples. Past work has indicated that, at least with manures, the presence of these bands has no significant effect on calibration accuracy or development (Reeves and Van Kessel, 2000a).

Unlike dairy manures, poultry litters can be expected to have significant amounts of uric acid present (Zublena et al., 1996). As demonstrated in Figure 5, uric acid (wet or dry) has a significant absorption in the region between 2,000 and 2,400 nm centered at about 2,210 nm, which is also where the absorption band for ammonia is. The presence of uric acid can therefore be expected to influence or interfere with calibrations for ammonia in the same samples.

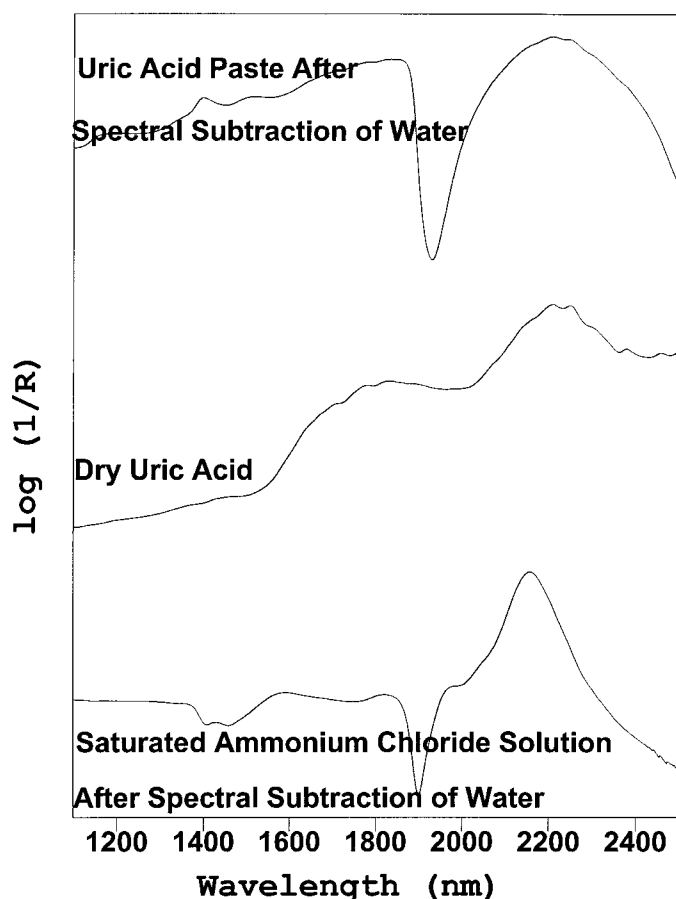


FIGURE 5. Near-infrared spectra of uric acid-water paste (water spectrum subtracted), of dry uric acid, and of saturated ammonium chloride solution (water spectrum subtracted).

Past work suggests that the bleach reagent used in the Quantofix can also react with other nitrogen-containing materials to various degrees (Van Kessel et al., 1999) including uric acid (unpublished data). Therefore, the spectra were examined for samples that the Quantofix measurement agreed and greatly disagreed with the reference ammonia levels (Figure 6). These samples had very different spectra in the 2,050 to 2,150 nm region but not at the main absorption peak for uric acid (2,210 nm). However, the sample with the least ammonia did show a peak at 2,210 (Figure 7). From these results, it is clear that the presence of uric acid could significantly alter spectra and thus result in potentially erroneous NIRS determinations, but it is not clear exactly what is really happening in the spectra of the poultry samples studied here.

NIR Calibration Results

Results using NIRS are presented in Figures 8 to 10. All results are based on as-is analyte values because calibrations using as-is data were always slightly more accurate than DM data. The results using all 136 samples for ammonia (Figure 8) indicate that NIRS-based determinations would be of value with a final R^2 of 0.90 and root mean squared deviation (RMSD) of ~10% of the mean

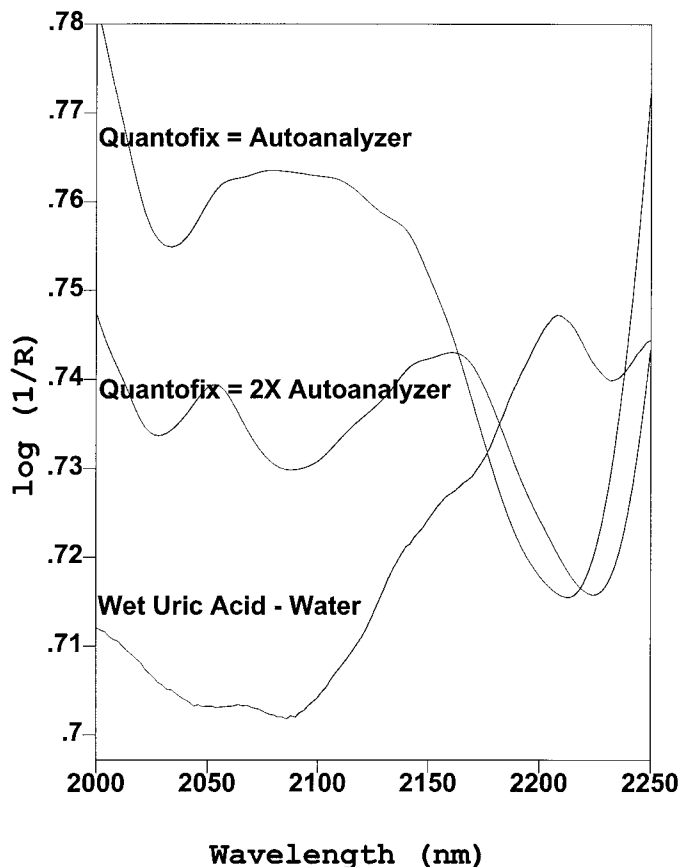


FIGURE 6. Partial near-infrared spectra of poultry litters, where Quantofix-NH₃ = reference NH₃, where Quantofix-NH₃ = twice reference NH₃; and of uric acid-water paste (water spectrum subtracted).

value. However, again these results are not quite as good as previously found with 124 poultry manures obtained from deep pits and floor scrapings ($r^2 = 0.95$, RMSD = 0.028 or ~6% of the mean; Reeves, 2001). Results with only the core samples were slightly better than those based on only the surface samples with R^2 of 0.93 and 0.88, respectively, for the core and surface samples. Although the number of samples available ($n = 68$) is marginal for these separate calibrations, the results indicate that the surface samples may change with time due to the sodium bisulfate or due to biological effects resulting in less interference with ammonia calibration development.

Finally, in Figures 9 and 10, results are presented for DM and conductivity. The results for DM determination were excellent ($R^2 = 0.98$, RMSD < 1% of the mean) and were about the same as previously found for the set of 124 poultry manures (Reeves, 2001), demonstrating that accurate calibrations were possible using the spectra available. The results for conductivity (Figure 10) were also very good ($R^2 = 0.94$, RMSD ~10% of the mean). Because inorganic salts do not have NIR spectra (Shenk et al., 1992), these results are difficult to explain. Because determinations of inorganic ions depend on their correlations with organic components, except for ammonia for which the N-H bands are observed, the conclusion can be

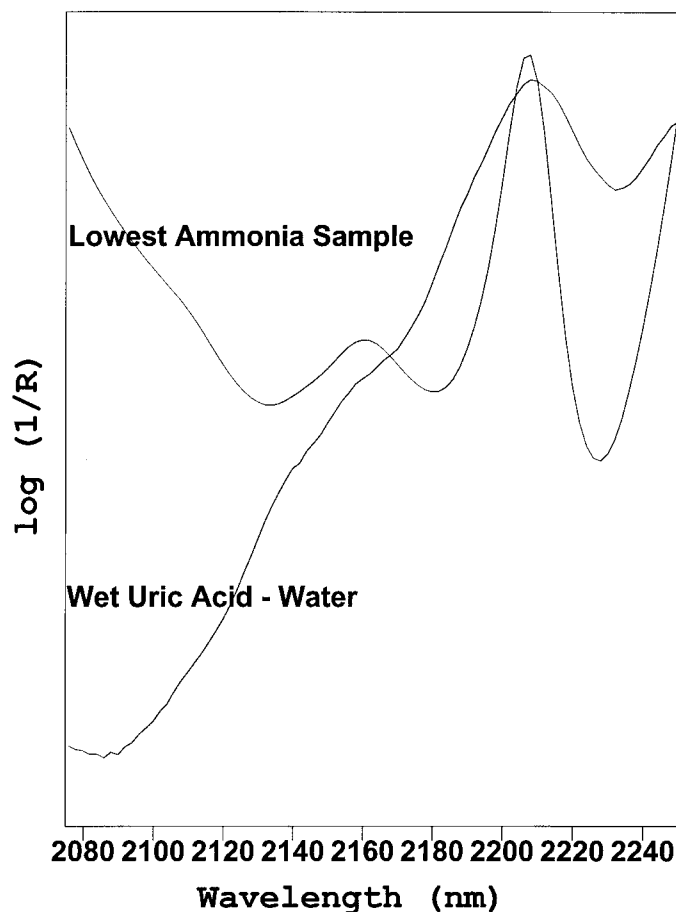


FIGURE 7. Partial near-infrared spectrum of poultry litters with lowest reference NH_3 content and of uric acid-water paste (water spectrum subtracted).

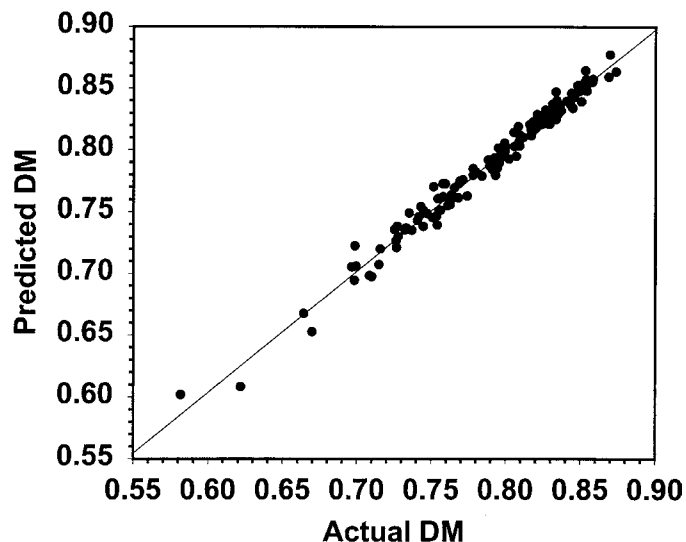


FIGURE 9. Final near-infrared calibration results for dry matter (%) with all 136 samples ($R^2 = 0.98$).

drawn that either ammonia or organic materials (perhaps uric acid) are being observed in the NIR spectra. The correlation between ammonia and conductivity for these same samples was only 0.26 on an as-is basis (r , not R^2 , = 0.07). Therefore, it appears that some organic compound is the likely basis for this calibration, with uric acid being a likely prospect.

Conclusions

Results showed that, although ammonia could be determined with reasonable accuracy by NIRS, using data from the spectral range of 1,100 to 2,498 nm (final calibration $R^2 = 0.90$), none of the QTS, including NIR, worked as well as previously found with dairy manures. The best

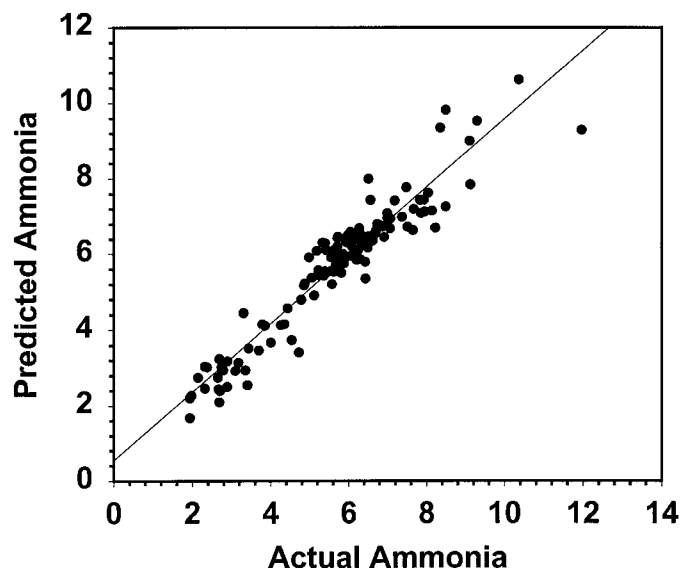


FIGURE 8. Final near-infrared calibration results for ammonia (mg $\text{NH}_4\text{-N/g}$ of sample) with all 136 samples ($R^2 = 0.90$).

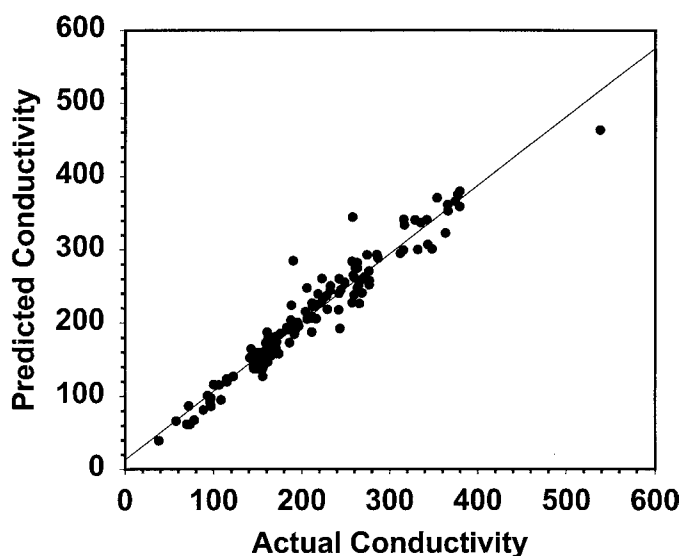


FIGURE 10. Final near-infrared calibration results for conductivity ($\mu\text{S/cm}$) with all 136 samples ($R^2 = 0.94$).

results were found using the Quantofix or Reflectoquant ($R^2 \sim 0.75$), and conductivity worked only with the core samples ($R^2 \sim 0.70$). It is believed that interference due to the presence of uric acid (spectroscopy, Quantofix determinations, and Reflectoquant) and sodium bisulfate that was used to treat the litter (conductivity determinations) are the cause of the problems incurred. Overall, from the results presented, it is apparent that the surface samples reacted differently than samples obtained from deeper in the litter (core samples). Although addition of sodium bisulfate to the litter can help explain the conductivity results, it is also apparent that other differences exist between the surface and core samples that the present data cannot explain. These differences could be the result of changes in organic fractions, including possibly uric acid, that occur as the litter ages. Further research may be useful to understanding the basis for the results in the present study, for modifying the current assays, or for developing new methods.

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